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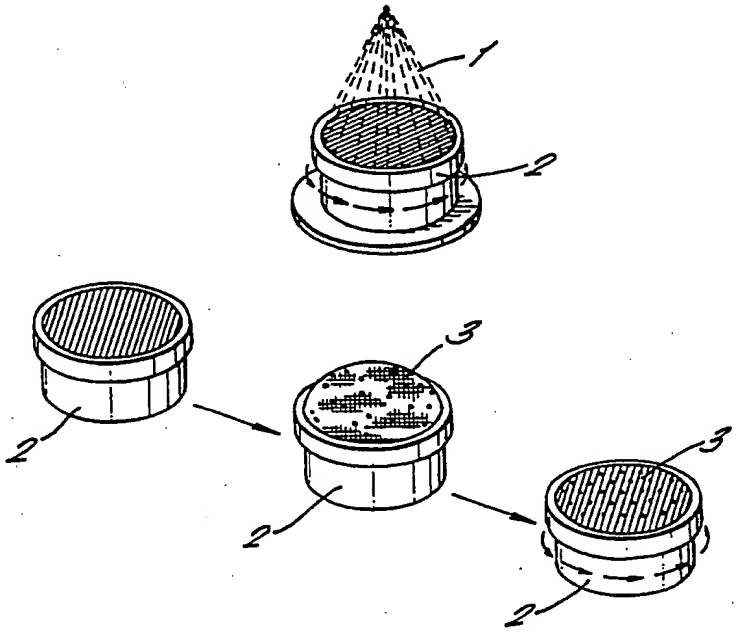
(54) Title: METHOD OF PREPARING A SAMPLE FOR ANALYSIS



(57) Abstract

A sample for analysis by Laser Desorption Mass Spectrometry is prepared by applying a substrate material to a target for the mass spectrometer, dissolving the sample material in a solvent and applying the solution to a substrate material on the target to be absorbed thereby. A matrix material is also dissolved in a solvent and this matrix material in solution is applied to the substrate material on the target. The solvent used to dissolve the matrix material is chosen such as to release the sample material from the substrate so that, after evaporation of the matrix material, there is provided on the substrate material an intimately mixed deposit of sample material and matrix material.

* See back of page

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METHOD OF PREPARING A SAMPLE FOR ANALYSIS

This invention relates to a method of preparing a sample for analysis, and particularly a sample for analysis by Laser Desorption Mass Spectrometry (LDMS) in which ions are sputtered from a condensed phase sample surface by photon bombardment and are then subjected to mass analysis.

Many methods of LDMS are known, and a feature common to many is the use of a matrix material in which the analyte (the sample material to be analysed) is dispersed. The matrix material can serve one or more of a plurality of functions. For example it may act as a mediator in transferring energy from the photon bombardment to the sample material molecules; it may provide a physical and chemical environment which enhances the probability of desorption in the desired state of charge and aggregation; and it may remove excess energy from the desorbed species through evaporation of matrix material molecules from a desorbed cluster of sample material and matrix material ions.

Four techniques for using a matrix material to enhance LDMS have been described as set out below.

The first is to dissolve the sample material together with a 10:1 excess of an inorganic salt in a solvent, place a drop of the solution on the target surface, and evaporate to

dryness as described by D.V. Davis et. al. in Analytical Chemistry, 55 1302 (1983). The sample material deposit is then irradiated with infra-red photons from a pulsed Neodymium YAG laser.

The second is to mix equimolar amounts of sample material and an inorganic salt in a droplet of glycerol placed on the target surface as described by L.G. Wright et. al. in Biomedical Mass Spectrometry, 12 159 (1985). The sample mixture is then irradiated with infra-red photons from a continuous wave carbon dioxide laser.

Thirdly, Japanese Patent Specification JP62-43562 discloses a sample preparation technique in which a solution of the sample material is mixed with a slurry of glycerol and fine cobalt powder. A droplet of the mixture is then irradiated with ultraviolet photons from a pulsed nitrogen laser.

Fourthly, M. Karas et. al. (Int. J. Mass Spectrom. Ion Processes, 78 53 (1987)) describe using a large molar excess of a matrix material which has a strong absorption at the wavelength of the incident radiation. For example, the sample material is dissolved in a solution containing a thousand-fold molar excess of Nicotinic Acid. A drop of the solution is placed on the target surface, evaporated to dryness, and irradiated with 266nm ultraviolet photons from a frequency quadrupled pulsed Neodynium YAG laser. The use of a matrix material which has a strong absorption for the incident photons represents an important distinction between this approach and

the first three described because it allows the use of low power densities which increases the probability of desorbing intact molecular ions.

According to this invention there is provided a method of preparing a sample for analysis by laser desorption mass spectrometry, comprising applying a substrate material to a target for the mass spectrometer to be used; dissolving the sample material in a solvent and applying the solution to the substrate material on the target to be absorbed thereby; dissolving a matrix material in a solvent; and applying the matrix material solution to the substrate material on the target, the solvent used to dissolve the matrix material being such as to release the sample material from the substrate material thereby to provide on the substrate material on the target, after evaporation of the matrix material solvent, an intimately mixed deposit of sample material and matrix material.

Preferably the matrix material has a strong absorption for the photon bombardment used for mass spectrometry.

Preferably the substrate material is applied to the target by a technique such as electrospraying which provides a deposit with a large surface area.

The invention will now be described by way of example with reference to the drawing, in which the two figures illustrate two methods of sample preparation in accordance with the invention.

Referring to Figure 1, a substrate material 1, for example Nitrocellulose, is electrosprayed in known manner onto the central region of a rotated target stage 2 of a mass spectrometer. A mask may be used to ensure that the matrix material 1 is restricted to a well defined area of known diameter. The electrospray technique is described fully by C.J. McNeal et. al. in Analytical Chemistry, 51 2036 (1979). A drop of sample material solution 3, for example a 10^{-5} molar solution of the peptide in 0.1% aqueous Trifluoroacetic Acid is placed onto the target 2 so as to cover the substrate material deposit. The sample material solution 3 is allowed to remain in contact with the substrate material for a period of several seconds so that the peptide molecules will bind to the Nitrocellulose through hydrophobic interaction. If the solution fails to wet the substrate material surface, a microscope slide cover slip can be placed on top of the droplet so as to encourage it to spread out over the surface. Once the peptide has been immobilised onto the substrate material surface, the droplet can be blown off with compressed gas, spun off, or rinsed off by either a stream of solvent or immersion in bulk solvent. Alternatively, the droplet could simply be allowed to dry out, but this would mean that any contaminants would also be left on the target surface.

The absorbed sample material solution can then be derivatised using a suitable chemical reagent or enzyme.

A droplet of matrix material solution consisting of

3×10^{-2} molar Nicotinic Acid in acetone is then applied to the target to cover the sample material deposit. The acetone solvent used for the matrix material will dissolve surface layers of the nitrocellulose substrate material together with the attached peptide molecules, so that sample material and matrix material become intimately mixed in the droplet. The droplet is then allowed to dry naturally by evaporation of the solvent (acetone) or can be assisted to dry by the application of heat or forced air.

The loaded target 2 can then be introduced into the source region of a mass spectrometer for analysis of the sample material by bombardment with 266nm photons from a frequency quadrupled Neodymium YAG laser, in known manner.

Substrate materials exhibiting highly specific binding properties such as immunoadsorbants can be used.

Referring now to Figure 2, an alternative method of loading sample material onto a target with the substrate material thereon is blotting. By this means, sample material may be transferred directly from an electrophoretic or chromatographic support onto a prepared target. The techniques for performing electrophoretic separations and staining are well known to those skilled in the art and descriptions may be found in "Electrophoresis, Theory, Techniques, and Biochemical and Clinical Applications" by A.T. Andrews, Clarendon Press, Oxford, 1986. The techniques for blotting are also well known and are described in "Protein Blotting, Methodology, Research

and Diagnostic Applications" edited by B.A. Baldo and E.R. Tovey, Karger, Basel, 1989.

In one embodiment, a mixture of proteins is denatured using sodium dodecyl sulphate and separated electrophoretically in a slab 21 of polyacrylamide gel. The gel is subsequently stained to reveal the positions of the separated components. The gel 21 is placed in a semi-dry blotting tank 22 on a filter paper 23 and a bottom electrode 24 and one or more targets 25 precoated with a substrate material are placed face down on the upper surface of the gel 21 at the locations of the components or interest. The filter paper 23 and the upper surface of the gel 21 are wetted with a solvent of 25mM Tris, 192 mM glycine, and 20% (v/v) methanol in water. By applying a potential difference of a few tens of volts from a source 26 between the bottom electrode 24 and the conductive targets 25, proteins are induced to migrate from the gel 21 towards the targets 25 where they are bound by the substrate material. The targets 25 may then be removed, rinsed, the matrix solution added as described previously, dried, and introduced into the source region of a mass spectrometer for analysis by bombardment with 266 nm photons from a frequency quadrupled Neodymium YAG laser.

CLAIMS

1. A method of preparing a sample for analysis by laser desorption mass spectrometry, comprising applying a substrate material to a target for the mass spectrometer to be used; dissolving the sample material in a solvent and applying the solution to the substrate material on the target to be absorbed thereby; dissolving a matrix material in a solvent; and applying the matrix material solution to the substrate material on the target, the solvent used to dissolve the matrix material being such as to release the sample material from the substrate material thereby to provide on the substrate material on the target, after evaporation of the matrix material solvent, an intimately mixed deposit of sample material and matrix material.

2. A method as claimed in Claim 1, in which the matrix material has a strong absorption for the photon bombardment used for mass spectrometry.

3. A method as claimed in Claim 1 or Claim 2, in which the substrate material is applied to the target by electrospraying.

4. A method as claimed in any preceding claim, in which the sample material is applied to the substrate material by blotting.

5. A method as claimed in any preceding claim, in which the substrate material is Nitrocellulose.

6. A method as claimed in any one of Claims 1 to 4, in which the substrate material is an immunoadsorbant.

7. A method as claimed in any preceding claim, in which the sample material is derivatised after application to the substrate material and before application of the matrix material solution.

8. A method of preparing a sample for analysis by laser desorption mass spectrometry, substantially as hereinbefore described with reference to Figure 1 or Figure 2 of the drawing.

FIG. 1.

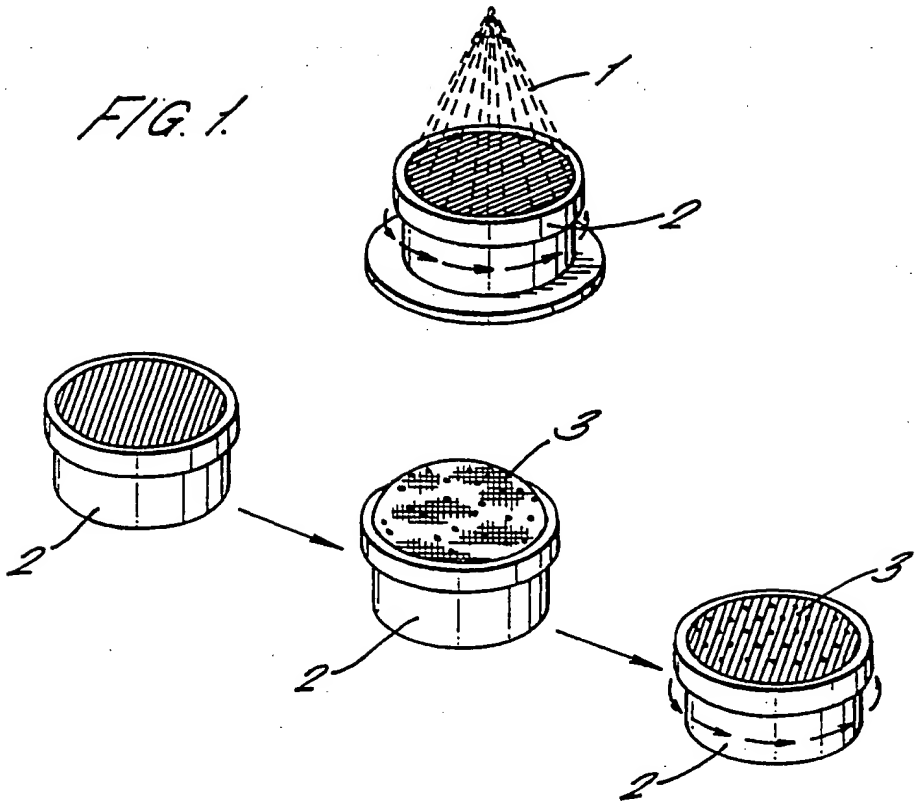
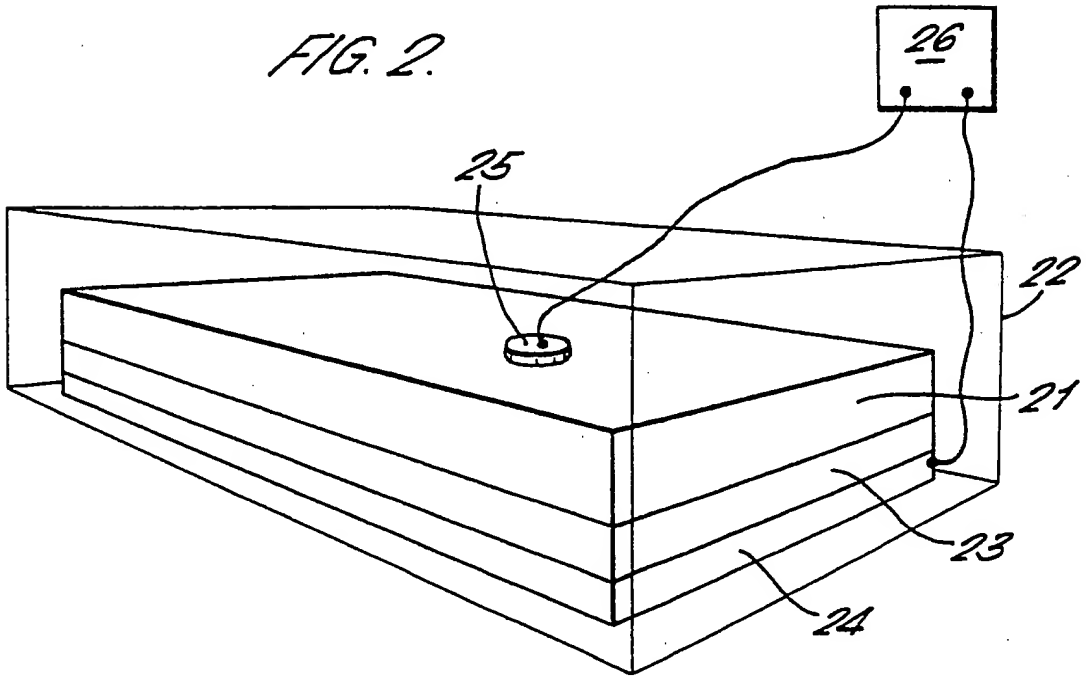


FIG. 2.



INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 90/00974

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC5: G 01 N 1/28, 27/62, H 01 J 49/04

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
IPC5	G 01 N; H 01 J

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category *	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
Y	Patent Abstracts of Japan, Vol 12, No 173, P706, abstract of JP 62-284256, publ 1987-12-10 (SHIMADZU CORP)	1-2
Y	WO, A1, 8701452 (HITACHI, LTD.) 12 March 1987, see page 4, line 23 - page 5, line 5; column 2, line 57 - line 63 in corresponding US-A-4874944	1-2
A	ANALYTICAL CHEMISTRY, Vol. 55, No. 8, July 1983, D.V. Davis et al: "Identification of Naturally Occurring Quaternary Compounds by Combined Laser Desorption and Tandem Mass Spectrometry ", see page 1302 - page 1305 especially page 1303, column 2, line 61 - line 69	1-2

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IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
25th September 1990	2[illegible] 10. 90
International Searching Authority	**Signature of Authorized Officer**
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III. DOCUMENTS CONSILERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
A	BIOMEDICAL MASS SPECTROMETRY, Vol. 12, No. 4, 1985, Larry G. Wright et al: "Matrix Enhanced Laser Desorption in Mass Spectrometry and Tandem Mass Spectrometry ", see page 159 - page 162 especially page 160, column 1, line 3 - column 2, line 19 --	1-2
A	Patent Abstracts of Japan, Vol 11, No 230, P599, abstract of JP 62- 43562, publ 1987-02-25 (SHIMADZU CORP) --	1-2
A	International Journal of Mass Spectrometry and Ion Processes, Vol. 78, 1987, M. Karas et al: "MATRIX-ASSISTED ULTRAVIOLET LASER DESORPTION OF NON-VOLATILE COMPOUNDS ", see page 53 - page 68 especially page 55, line 1 - line 18 --	1-2

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. PCT/GB 90/00974

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This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 28/08/90 The European Patent office is in no way liable for theseparticulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A1- 8701452	12/03/87	GB-A-B- 2187327	03/09/87
		JP-A- 62051144	05/03/87

For more details about this annex : see Official Journal of the European patent Office, No. 12/82

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Ciphergen Announces Advances in ProteinChip® Proteomics

Fremont, CA, May 21, 2001 – Ciphergen Biosystems Inc. (NASDAQ:CIPH) announced today at the 49th Annual Conference on Mass Spectrometry and Allied Topics in Chicago an expanded product portfolio to address the needs of both clinical and basic research proteomics laboratories. Ciphergen introduced;

- a new SELDI (surface enhanced laser desorption/ionization) ProteinChip interface to high-end quadrupole time-of-flight tandem mass spectrometers that enables 'on-chip', high performance protein identification, epitope mapping and protein interaction analyses.
- new Biomarker Patterns™ software which enables pattern recognition analysis of protein expression profiles generated by its ProteinChip System and Arrays.
- new high performance ProteinChip Arrays which feature state-of-the-art latex-based polymer surfaces that provide greatly improved selectivity, sensitivity and reproducibility for protein expression profiling applications.
- new ProteinChip Arrays which have been packaged into a series of application-specific kits to enhance ease-of-use for the biologist performing protein analysis.

" These new products provide significant improvements in protein expression profiling, protein interaction and protein identification applications, said Richard B. Rubin, Director of Marketing for Ciphergen. "Combined with our ProteinChip System, the new SELDI interface for tandem MS will complement both affinity LC-MS and 2-D gel methods used in basic research and drug discovery proteomics." Commenting on the new tandem MS interface program, Dr. William E. Rich, President and CEO of Ciphergen said, "We've successfully demonstrated the utility of the ProteinChip-Tandem MS interface in several projects in our Fremont Biomarker Center over the past year. ProteinChip-Tandem MS capability works in harmony with our existing technology allowing you to take a sample that's been profiled 'on-chip' directly to the tandem MS for peptide fragmentation-based sequence analysis."

We believe clinical proteomics, pioneered by Ciphergen, will particularly benefit from the new pattern recognition software. The Biomarker Patterns Software module that is being launched addresses a key component of the biomarker discovery process. A major benefit of the ProteinChip platform is in the discovery and correlation of multiple biomarkers in a population of samples. As was the case in the development of DNA array technology, the flood of data produced by the instrument makes informatics tools critical to interpreting the results. The new software package combined with an updated 'Biomarker Wizard' in Ciphergen's core software package automatically identifies multiple protein peaks that correlate with differences between samples. The new line of ProteinChip Arrays improve both the total number of proteins detected from a single sample and the data reproducibility from run-to-run making the ultimate sample stratification results achieved by the pattern software more reliable.

Ciphergen also announced a new line of ProteinChip Kits making it easier for new users of the system to adopt the technology and achieve reliable results. The first kits to be released address key applications including protein profiling, peptide mapping, antibody capture and a beta-amyloid multipeptide assay for Alzheimer's research. These kits consist of specific arrays, reagents and an optimized protocol.

Ciphergen develops, manufactures and markets the ProteinChip System that enables protein discovery, characterization and assay development to provide researchers with a better understanding of biological functions at the protein level. The ProteinChip System is a novel, enabling tool in the emerging field of protein-based biology research, known as proteomics. Proteomics provides a direct approach to understanding the role of proteins in the biology of disease, monitoring of disease progression and the therapeutic effects of drugs. Ciphergen believes proteomics will be a major focus of biological research by enhancing the understanding of gene function and the molecular basis of disease.

Note Regarding Forward-Looking Statements: This press release contains forward looking statements, including statements regarding Ciphergen's plans to exploit differential protein expression analysis. Actual results may differ materially from those projected in such forward-looking statements due to various factors, including Ciphergen's ability to discover and then validate biomarkers that have applications in pre-clinical and clinical drug development. Investors should consult Ciphergen's filings with the Securities and Exchange Commission, including its Form 10-K for the year ended December 31, 2000, for further information regarding these and the other risks of the Company's business.